

Challenging the inflammatory response system: Are individuals with insomnia more reactive?

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PART B

STUDY DESCRIPTION

TITLE OF PROTOCOL	Challenging the inflammatory response system: Are individuals with insomnia more reactive?
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B1. PURPOSE OF PROTOCOL

Primary objective: To investigate the inflammatory response to a physiological stress challenge in insomnia disorder. We expect that individuals with insomnia are more reactive, as manifested in a stronger response of inflammatory systems markers (interleukin [IL]-6, prostaglandin [PG]E₂) to a physiological stress challenge (cold pressor test). In accordance, we expect a stronger autonomic (blood pressure, heart rate) and hypothalamus-pituitary-adrenal (HPA, cortisol) response to challenge in insomnia disorder compared to healthy controls.

Secondary objective 1: To investigate the ability to habituate to a repeated physiological stress challenge. We expect that the process of habituation is deteriorated in insomnia compared to healthy controls, as indicated by the lack of the inflammatory, autonomic, and HPA responses to decrease upon repeated exposure to the cold pressor test.

Secondary objective 2: To investigate whether the inflammatory response to challenge can be predicted by the sensitivity of immune cells to glucocorticoids. We expect that in insomnia, monocytes are less sensitive to the counter-inflammatory signal of cortisol, resulting in an exaggerated inflammatory response to a physiological stress challenge.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Sleep is critical for the regulation and maintenance of biological systems, and sleep deficiency, such as insomnia, has been shown to be associated with elevated risks for cardiovascular, metabolic, and mood disorders¹¹.

Despite the high prevalence of insomnia in the population²³, our understanding of the biological consequences of the disorder with respect to inflammatory, autonomic, and HPA system markers is limited, and often not consistent. For example, changes in the inflammatory system have been indicated by findings of increased levels of interleukin-6 (IL-6)^{5;33}, but not C-reactive protein (CRP)²⁰. Changes in the sympatho-adrenal system, as indicated by elevated blood pressure levels or increased incidence of hypertension^{10;19}, and increased circulating norepinephrine levels¹⁷ have been reported in several studies. HPA stress system marker, as measured by cortisol levels in serum or plasma, have been found increased in insomnia in some studies^{26;31}, but not others^{25;30}.

All these investigations on the impact of insomnia on biological system markers have in common that they measure **basal** levels of various system markers. However, **insomnia may not only alter the basal activity of these systems, but may change their reactivity to other stressors and challenges**. In support of this assumption are findings showing that poor sleep quality in healthy individuals is associated with a stronger IL-6¹⁶, cortisol¹³, and heart rate³² response to a stressful challenge, such as the cold pressor test (CPT). The CPT involves the immersion of the hand in ice-cold water kept at about 3C for an interval between 30sec to 3min. It is one of the most commonly used laboratory physiological challenge tests, provoking not only unpleasantness, but also increases in cortisol, norepinephrine, and inflammatory cytokines within 15-50min post-testing^{18;27;35}.

Investigations of such system's reactivity to challenge may elucidate systems abnormalities that we do not capture by only assessing basal system's levels. For example, in patients with rheumatoid arthritis, basal IL-6 levels are normal, but the response to a physiological stress challenge (cold pressor test) is amplified⁹. Similarly, individuals with poor sleep quality cannot be distinguished from good sleepers by their basal IL-levels, only by their IL-6 reactivity to challenge¹⁶.

However, to our knowledge, no studies have measured how insomnia may affect the reactivity of biological systems to a stressful challenge, which may serve as an important indicator of system's dysregulation and associated disease risk²¹

In this light, the **primary objective of this proposal will investigate whether the inflammatory, autonomic, and HPA systems are more reactive to a physiological stressful challenge in insomnia disorder compared to healthy controls.**

Beside the hypothesized changes in the system's response reactivity in insomnia, the **secondary objective 1 of this proposal will investigate the ability of system's responses to habituate to repeated stressful challenges.** Habituation, or a decreased response following repeated exposures to a stressful challenge, is a key feature of the adaptive nature of many biological systems, and has been observed in response to a variety of psychological and physiological stressors and challenges in both humans and animals¹⁵. Considering that most individuals have to deal with stressful challenges on a daily basis, failure of systems to habituate to such daily challenges may elevate disease risk in the long term¹⁵. To test the degree of habituation, we will repeatedly administer the CPT and measure the inflammatory, autonomic, and HPA response magnitude after each challenge.

In summary, this proposal focuses on system's reactivity to challenge (primary objective) and the ability of systems to adapt to repeated challenges (secondary objective 1), rather than assessing basal systems activity only. Dysregulation of systems reactivity and habituation are likely to constitute processes that may serve as valuable predictors of disease risk in insomnia.

Secondary objectives 2 and 3 investigate potential determinants of the inflammatory response to challenge. **Is the proposed stronger inflammatory response to challenge in insomnia due to less sensitivity of immune cells to the counter-inflammatory actions of cortisol (secondary objective 2)?** One potential mechanism contributing to increased inflammatory reactivity is a change in the sensitivity of monocytes to the glucocorticoid (GC) cortisol. Indeed, impaired GC sensitivity has been reported in response under various medical and/or stressful conditions^{24;28}, facilitating synthesis and release of inflammatory cytokines by monocytes. **Though insomnia disorder has been frequently associated with elevated cortisol levels², the sensitivity of immune cells to the cortisol signal surprisingly has never been assessed.** Thus, the secondary objective aim 2 will investigate whether GC sensitivity in insomnia is a potential predictor of the proposed inflammatory response increase to challenge.

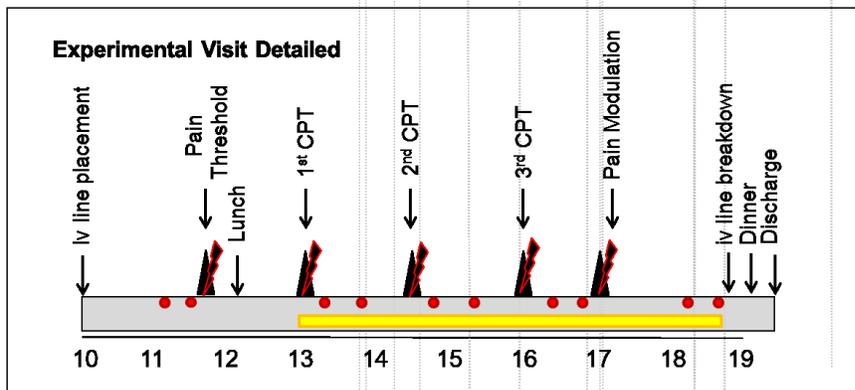
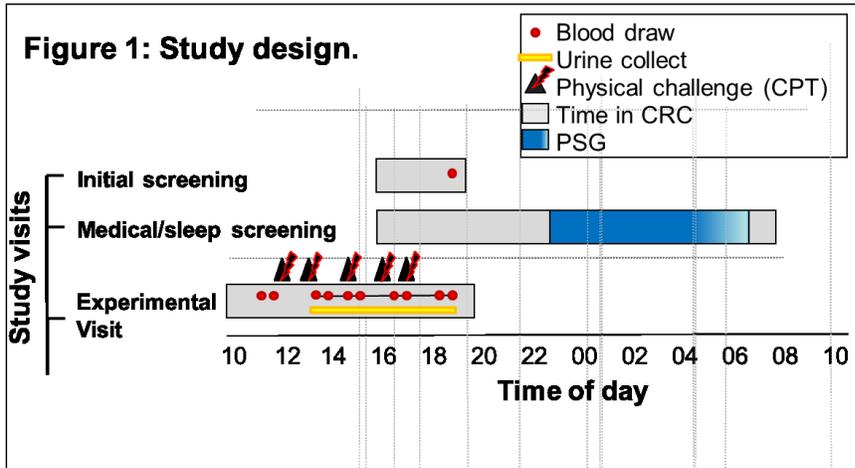
To conclude, this proposal two important concepts that have not been addressed in insomnia: Response reactivity to a stressful challenge and the ability to habituate to repeated exposure of such challenge. These two concepts may serve as critical indicators of disease risk in the long term^{15;21}. In addition, we will investigate a potential determinant of inflammatory reactivity: GC sensitivity that may serve as target for future drug development and evaluation.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

As seen in the **Figure 1**, the research design consists of an initial screening visit, followed by an in-hospital overnight medical/sleep screening. If the participant meets the study recruitment requirements they will immediately begin the 1 day in-hospital study visit.

Visit 1. Initial screening. All potential participants will have the study explained to them in detail by the PI or another senior member of the research team. If interested, potential participants will sign the informed consent and nursing staff in the Clinical Research Center (CRC) will take vital signs and collect a blood sample. A short battery of screening tests and questionnaires will also be collected in order to assess health status and ability to tolerate ice-cold temperatures (a water bath) without adverse circulatory responses. In addition, participants will be asked to record their sleep in the home environment, using sleep diary and actigraphy.



Visit 2. Medical history, physical, and sleep screening.

During this visit, urine toxicology and pregnancy (for women) tests will be performed, and the study physician will meet with the study participant for a physical exam and review of blood test results. Participants who remain eligible following medical screen will be set up with PSG equipment for a sleep screening night in the CRC, in order to rule out any possible sleep disorders.

If a participant has done a screening overnight sleep study within the last 6 months, or a sleep diary recording, screening blood test (including lipid panel, insulin, glucose, TSH, CBC, PLT, CRP, sodium, potassium, bilirubin, creatinine, ALT, absolute T Cells, sediment rate), or had a physical examination within the last 3 months, these results will be used for this research.

1-Day CRC Visit: This visit will be scheduled directly following the medical/PSG screen. At 10am, an intravenous (iv) line will be placed for blood drawing. Pain threshold measures (heat and pressure) will be assessed about an hour later. Baseline assessment will begin at 1am lasting until 12pm, during which blood (inflammatory/HPA markers), urine (prostaglandin/norepinephrine), and autonomic variables (beat-to-beat blood pressure, heart rate) will be collected. The pain threshold testing will start at 1100am. Following the procedures the heat pain threshold will be assessed by a computer controlled thermode (Somatosensory Analyzer, Medoc, Minneapolis, MS) will be placed on their

forearm. The participant will click a mouse when they feel the thermode temperature change from neutral to warm and then hot to painful. The pressure pain threshold will be measured by electronic pressure algometer (Somedic Sales AB, Hörby, Sweden. Participants will press a control button when they experience the first sensation of pain. The reactivity and habituation assessment will take place at 1pm. The cold pressor test (CPT) will be administered 3 times. As detailed under procedures, participants will be instructed to immerse their hand in ice-cold water kept at 3 degree C, for a duration of 1min. the interval between the three cold pressor tests will be 1.5 hours. Blood will be sampled after the 1st, 2nd, and 3rd cold pressor test twice, 20min and 50min after hand removal from the cold water bath (these timepoints have been shown sensitive to capture increases of cortisol and IL-6). Urine will also be collected during and after the administration of the cold pressor test series, starting at 1pm and closing out at 6pm.. Prior to, during, and after the cold pressor series, participants will rate their stress levels as well as the unpleasantness of the cold sensation induced by the CPT. The final pain modulation tests, temporal summation and conditioned pain modulation will occur at 500pm. Temporal summation of pain is frequently used as an index of central sensitization of dorsal horn neurons⁵⁶, and will be assessed using the Somatosensory Analyzer (Medoc, Minneapolis, MS). The participant will have the thermode placed on forearm volar skin of the arm not used for blood draws. Four sequences each consisting of ten brief consecutive heat pulses will be applied via a thermode with a pulse-to-pulse interval of 2.5 sec. Conditioned pain modulation assesses the principle of 'pain inhibits pain', and is a measure of the pain-inhibitory capacity of the central nervous system. The participant will have the thermode placed on the forearm volar skin not used for blood draws..For the test, *the test stimulus* will be the TS sequence at highest tolerable temperature, applied to the outer volar surface of the non-dominant forearm, along C8-T1 innervations. Immersion of the contralateral foot into a painfully hot water bath (47 °C, Techne® water baths, Bibby Scientific US, Burlington, NJ) Prior to discharge, participants will receive a dinner shortly after 6pm.

Procedures:

Polysomnographic recording (PSG). Sleep will be recorded using the Embla system N7000 (Medcare US, Buffalo) on the screening night visit to ensure that participants are free from sleep disorders (clinical interview will be used to diagnose insomnia disorder and also to support the diagnoses of other sleep disorders, such as nightmare disorder, circadian rhythm disorder).

Blood Sampling. Blood samples will be drawn at intermittent intervals during the experimental visit using an indwelling 18-gauge forearm catheter from 1pm to 7pm. The total amount of blood taken will not exceed 200ml.

IL-6 will be measured in plasma in our laboratory, using a high sensitivity enzyme immunosorbent assay (ELISA, Quantikine ® HS, R&D Systems, Minneapolis, MN). IL-6 will be measured at baseline, after each cold pressor test, and hourly after the last cold pressor test until 7pm.

PGE2 metabolite will be measured in urine collected during the cold pressor test series (from 3-7pm). Urinary PGE2 will be analyzed in our lab using an EIA (Cayman Chemical, Ann Arbor, MI), and levels will be adjusted for urinary creatinine levels.

Cortisol will be measured in plasma and assayed in the Harvard Catalyst Central Lab, using the Access Chemiluminescent Immunoassay (Beckman Coulter Fullerton, CA). Cortisol will be measured at baseline, after each cold pressor test, and hourly after the last cold pressor test until 7pm.

Glucocorticoid (GC) sensitivity will be assessed in vitro by examining the capacity of monocytes to produce IL-6 in response to the synthetic glucocorticoid dexamethasone (DEX). In brief, whole blood will be incubated with LPS (100 pg/ml) in the presence of different concentrations of DEX (0-400nM). After incubation with fluorescence-conjugated antibodies (CD14, CD45, IL-6), monocyte expression of IL-6 will be measured in the Flow Cytometry Core at the BIDMC (Gallios™, Beckman-Coulter Inc). This protocol was developed by the PI for our currently ongoing study on pattern of repeated sleep restriction and recovery (R01 HL 105544). Lower IL-6 expression in response to DEX would indicate

a higher GC sensitivity and this mechanism may contribute to the proposed stronger inflammatory reactivity in insomnia disorder. Measures will be performed once at baseline, using the 2pm blood.

Autonomic markers: Blood pressure (BP) measurements will be collected during baseline, throughout the testing sessions and recovery period of the experimental visit, using the dinamap system.

Physiological stress challenge – cold pressor test (CPT): A trial CPT will be administered during the initial screening to identify any previously-unrecognized incidence of Raynaud's phenomenon or inability to tolerate the ice bath. The CPT will be administered three times during the experimental visit, starting at 1pm. Throughout the testing and recovery period (until 6pm), the participant will remain in a seated position in a comfortable chair. The CPT will be performed using the hand/arm that is not being used for blood sampling in order to prevent interference. A series of three CPT tests will be applied with an inter-test interval of 60min. Repeated administrations of the cold pressor test have been performed in previous studies (with intervals as short as 2min^{8;13}), but without investigating system's responses. For the current study purposes, an inter-test interval of 60 min has been chosen to capture response increases of IL-6 and cortisol^{8;9;16}. For each CPT test, participants will be asked to insert their hand in a temperature-controlled water bath (Techne® water baths, Bibby Scientific US, Burlington, NJ), kept at 3 degree C, and instructed to leave their hand in for a duration of 1min (participants are told that they can take their hand out earlier if the sensation is getting unbearable). Blood will be drawn 20 and 50min after hand removal from the cold water bath. These time points have been shown sensitive to capture peak cortisol levels as well as significant IL-6 increases^{8;9;16}. Participants will rate the unpleasantness of the sensation prior to hand immersion into the cold water bath, and continue with ratings at 10sec intervals during and after the testing period (up to 3min post-testing).

Pain threshold testing details:

- (a) **Heat pain thresholds (HPT)** will be assessed using a precise, computer-controlled thermode (Somatosensory Analyzer, Medoc, Minneapolis, MS) for generating and recording responses to thermal stimuli. The thermode will be attached to the forearm volar skin of the arm not used for blood draws, via a Velcro strap, to the palm. From a baseline temperature of 32°C, the thermode is heated at a rate of 0.5 °C/sec. The participant is instructed to click a computer mouse with their free hand, as soon as heat pain is perceived. To improve accuracy, each threshold will be measured 4 times. The inter-stimulus interval is 15 sec, and the average will be used for further analysis.
- (b) **Pressure pain thresholds (PPT)** will be obtained with use of an electronic pressure algometer (Somedic Sales AB, Hörby, Sweden), and assessed at the posterior neck and trapezius muscle, and pressure will be increased at a rate of 30 kPa/s. Participants will be instructed to press a control button when they experienced the first sensation of pain. A series of four pressure-pain stimuli will be applied with 15 sec intervals to each side, and the average of these stimuli will be calculated.

Modulation testing details:

- (a) **Temporal summation (TS)** of pain is frequently used as an index of central sensitization of dorsal horn neurons⁵⁶, and will be assessed using the Somatosensory Analyzer (Medoc, Minneapolis, MS). Four sequences each consisting of ten brief consecutive heat pulses will be applied via a thermode to the forearm volar skin of the arm not used for blood draws, with a pulse-to-pulse interval of 2.5 sec. The temperature used to assess TS will be tailored to each person's tolerance level. The first test sequence of this individually-tailored procedure will have a target temperature of 48 °C and an inter-pulse baseline temperature of 32 °C. Depending on whether the participant can tolerate the initial 10-pulse sequence, the target temperature of the second sequence will be increased or decreased, respectively, by 1.5 °C (i.e., increased to 49.5 °C or decreased to 46.5 °C). In the third sequence, the target temperature will be again increased or decreased by 1.5 °C, depending on whether the participant could tolerate the second sequence or not. The inter-sequence interval will be 2 min at a temperature of 32 °C. The thermode will be moved systematically between sequences, starting at the thenar eminence for a practice trial, and sequentially moving cephalad on the volar aspect of the

forearm along the innervation of C5–6 for the remaining three trials. This sequence was designed to prevent testing on previously stimulated skin areas (see also²⁵). During each test sequence, participants will be prompted to rate the intensity of the 1st, 4th, 7th, and 10th thermal pulse using a 0–100 mm visual analogue scale (VAS), presented on separate data sheets for each rating. The participant will be instructed to say ‘STOP’ as soon as the sensation is no longer tolerable at any point during the testing.

- (b) **Conditioned pain modulation (CPM)**, assesses the principle of ‘pain inhibits pain’, and is a measure of the pain-inhibitory capacity of the central nervous system. For this protocol, *the test stimulus* will be the TS sequence at highest tolerable temperature, applied to the outer volar surface of the non-dominant forearm, along C8-T1 innervations. Immersion of the contralateral foot into a painfully hot water bath (47 °C, Techne® water baths, Bibby Scientific US, Burlington, NJ) will be the *conditioning pain stimulus* that is intended to activate the pain-

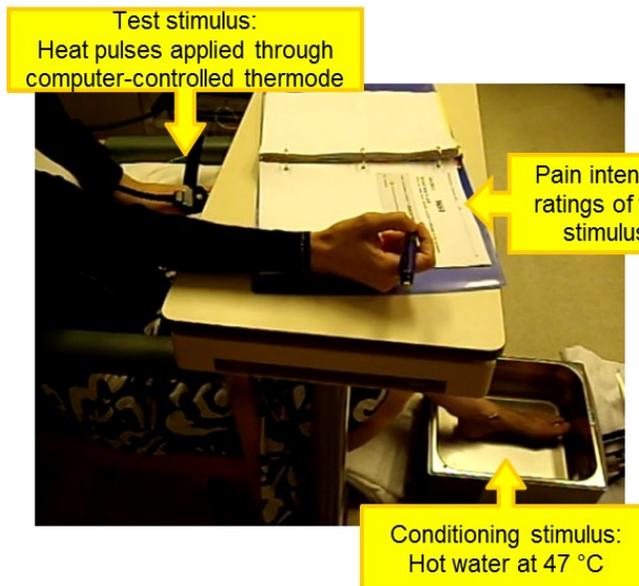


Figure 2: Setup for Conditioned Pain Modulation (CPM) testing:

Twenty sec after foot immersion into hot water bath (47 °C, the conditioning stimulus that is supposed to activate descending pain-inhibitory circuits), the participant rates the intensity of heat pulses (test stimulus, individually tailored to highest tolerable temperature) applied to the forearm.

inhibitory circuits and thereby decrease the perceived pain of the forearm test stimulus (see **Figure 2** for setup).

In total, four CPM trials will be performed: two trials using a hot water bath (47 °C) and two baseline trials without a water bath. For each hot water bath trial, the participant’s foot will be first submerged in the water bath. After 20sec of immersion, the 10-pulse temporal summation sequence will be applied to the forearm and the participant will be prompted to rate the pain intensity of the 1st, 4th, 7th, and 10th stimulus using the VAS. There will be a two-minute rest period between all trials during which the thermode will be systematically moved from the distal to proximal sites along the C8-T1 innervated skin in order to avoid re-stimulation of the previously sensitized skin. For statistical analysis, ratings of the two 10-pulse sequences under the conditioning stimulus (hot water) will be averaged, and contrasted against ratings from the baseline (no-water) condition. A decrease in pain intensity ratings under the hot water condition compared to the no-water condition will be used as an index of the pain-inhibitory capacity.

B. Statistical Considerations

Sample Size Justification: For sufficient statistical power, we will need to have 20 participants with a diagnosis of insomnia disorder (based on DSM-V criteria), and 20 healthy control sleepers complete the study protocol (60% women, see power calculation below).

Power calculation

Power calculations were performed for the primary objective of this proposal. With respect to the proposed stronger **inflammatory response reactivity** in insomnia compared to healthy controls, power calculations were based on findings from Heffner et al.¹⁶, reporting a difference in the IL-6 response between poor and good sleepers to an acute challenge of 1.6 pg/ml (SD 1.71 pg/ml, estimated from graph). Thus, we need to study **20 participants in each group** to reach optimal power of 80% (unpaired t-test, alpha = 0.05).

With respect to the proposed stronger **cortisol response reactivity** in insomnia compared to healthy controls, power calculations were based on findings from Goodin et al.¹⁴, reporting a difference in the cortisol response between poor and good sleepers to an acute challenge (CPT) of 10 units (area under the curve, SD 11 units, estimated from graph). Thus, 80% power will be reached with a sample size of N=20 per group

Statistics: Primary objective: Testing response reactivity to a physical challenge. The overall statistical analysis will be performed within the frame of mixed linear models. Primary and secondary outcome variables pertaining to the primary objective (see above) will constitute the independent variable, time (baseline, 1st CPT post-testing) and group (insomnia vs. healthy controls) will be entered as fixed factors, while subject number will serve as random factor. Simple contrasts will be performed between groups to identify differences at single time points.

Secondary objective 1: Testing response habituation to a repeated physical challenge. Data analysis will be similar as for the primary objective, but the time element will be presented by measures at baseline, 1st CPT post-testing, 2nd CPT post-testing, and 3rd CPT post-testing.

Secondary objective 2: GC sensitivity as a predictor of inflammatory response reactivity. Univariate analysis will be used to identify differences between insomnia and healthy controls in GC sensitivity at baseline. Regression analysis will be used to predict inflammatory response reactivity.

Secondary objective 3: Actigraphy-based sleep characteristics as predictors of inflammatory, autonomic, and HPA response reactivity. Univariate analysis will be performed to identify differences in sleep variables (as mentioned under secondary outcome variables above). Regression analysis will be used to predict response reactivity.

For all analysis, alpha will be set to 0.05.

C. Subject Selection

Human Subjects Involvement and Characteristics

A total of 40 healthy adults (18-55 years, not including attrition rate of 10%) will be enrolled. 20 participants with the diagnosis of insomnia disorder (DSM-V), 20 age- and gender matched healthy controls. 60% women (equivalent to an insomnia risk ratio of 1.4 in women versus men) Subjects of all racial/ethnic backgrounds will be eligible to participate. Participants will be accepted into the study if they are in good health, do not have a history of any medical disorders (including sleep disorders), as determined by a medical history, physical screening, and overnight sleep study (PSG). Participants from the Boston area, who meet these strict medical eligibility criteria, will be recruited to participate in the study. The study physician will also perform an in-person visit with each participant on day that they are in-hospital, in order to ensure patient's well-being.

Inclusion Criteria:

- Women and men between the ages 18-55 years.
- Body mass index (BMI) ≤ 35 .
- Blood chemistry in the normal range

Specific to **insomnia group**:

Diagnosis of insomnia based on DSM-V criteria¹:

- A. A predominant complaint of dissatisfaction with sleep quantity or quality, associated with one (or more) of the following symptoms: difficulty initiating sleep, difficulty maintaining sleep, early-morning awakening with inability to return to sleep.
- B. The sleep disturbance causes clinically significant distress or impairment of functioning (social, occupational etc.).
- C. The sleep difficulty occurs at least 3 nights per week.
- D. The sleep difficulty occurs despite adequate opportunity for sleep.
- E. The insomnia is not better explained by and does not occur exclusively during the course of another sleep-wake disorder.
- F. The insomnia is not attributable to the physiological effects of a substance (e.g., a drug of abuse).
- G. Coexisting mental disorders and medical conditions do not adequately explain the predominant complaint of insomnia.

Specific for **control group**:

- Good quality and quantity sleep (as determined by sleep diary and actigraphy: 7 to 9 hours of sleep duration/night, sleep onset latency (SOL) and wake after sleep onset (WASO) < 20 min/night).
-

Exclusion Criteria:

- Active infection/disease.
- History of neurological, chronic pain, immune, cardiovascular, liver/kidney, or metabolic disorder within the last 6 months prior to study start.
- History of psychiatric disorders in the last 6 months prior to study start, including major depressive disorders, bipolar disorders, panic disorders, post-traumatic stress disorders (PTSD), thought disorders, and substance abuse/dependence disorders.
- Sleep disorders other than insomnia: Apnea Hypopnea Index of > 15 events/hour on polysomnographic sleep study, periodic leg movement index (PLMI) > 10 /hour; sleep efficiency $< 80\%$ (only for control participants) based on polysomnographic screening night; restless legs syndrome, circadian rhythm disorders, and nightmare disorders determined by diagnostic interview.
- Reynaud's disease.
- Psychotropic, sleep, or any other medications or herbs interfering with the inflammatory,

autonomic, or HPA system in the last 2 weeks prior to study start, except oral contraceptives.

Note 1: Individuals who are currently using medication will be invited to undergo a taper with permission from, and supervised by, their physician and will be eligible for participation 2 weeks following cessation of all medication use.

Note 2: Information on historical medication use (prior to the 2-week medication-free period before study start) will be collected and incorporated into the statistical analysis plan if group differences exist.

- In psychotherapy or any other behavioral interventions at study start (e.g., acupuncture for insomnia).
- Pregnant/nursing.
- History of psychiatric, neurological, pain-related, immune, gastrointestinal, or cardiovascular disease; liver/kidney or metabolic disorder within the last 6 months prior to the study start..
- Esophageal reflux; gastric or duodenal ulcers; or asthma
- Pregnant/nursing.
- Regular medication use other than oral contraceptives.
- Donation of blood or platelets 3 month prior to or in-between in-hospital visits.
- Substance abuse.

Participants with slightly abnormal blood values will be included if they are not clinically meaningful.

Results from the toxicology screen will not be used as eligibility criteria for study continuation, but will be used for data quality control purposes.

Sources of Materials:

Blood and urine samples from participants will be assayed for markers involved in the stress response.

Inclusion of Women:

All women of child bearing age who do or do not take oral contraceptives and who are not pregnant or nursing are eligible for the study.

B4. POSSIBLE BENEFITS

This research will not benefit participants directly. However it will provide important information regarding our understanding of the biological consequences on insomnia disorder with respect to cardiovascular, metabolic and mood disorders.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

There is a minimal risk of infection associated with drawing blood from a vein. On rare occasions, pressure pain testing can cause bruising at the site where the pressure has been applied. The application of heat pain can lead to sensitization of the skin for up to 48 hours. There are no known risks of cold pressor test. There are no known risks of wearing BP or PSG equipment.

Participants will be extensively screened before being accepted into the study. We will exclude participants with a history of immune, cardiovascular, pain-related, gastrointestinal, significant psychiatric disease, significant allergies, or conditions where extreme vasoconstriction can occur, such as Raynaud's syndrome. Participant screening will include blood tests for CBC and differentials, as well as thyroid hormone, and blood glucose; urinary toxicology screens will also be conducted. We will exclude participants who smoke or take regular medications other than hormonal birth control medication. The medical history and physical will be conducted by the study physician. The study physician will check on all participants while they are admitted to the CRC, and serve as the doctor on-call throughout each participant's stay. Should participants need medical assistance, the monitoring research assistant will notify the ward nurses, who will address any immediate medical needs and also immediately notify the study physician and the PI. In the event of a medical emergency, the CRC is located in a critical care hospital, and a code team is available at all times.

Risk/benefit ratio: This work has the potential to understand how insomnia disorder may affect the reactivity of biological systems to a stressful challenge, which may serve as an important indicator of the system's dysregulation and associated disease risk.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

Participants will be recruited via internet postings, flyers, bulletin advertisements, and local newspapers. When participants contact the research office, they will participate in preliminary screening via telephone and/or email. If it appears that an individual may qualify to participate in the study, s/he will be invited to come to the Clinical Research Center (CRC) where s/he will go through the informed consent process and complete questionnaires designed to assist in determining eligibility. During the initial visit to the CRC, nurses will also take vital signs and collect a blood sample. These results will be reviewed by the study physician prior to the potential participants' 2nd CRC visit, during which participants will undergo a medical history/physical and overnight sleep screening. If an eligible individual is still interested in study participation, s/he will be scheduled to participate in the in-hospital portion of the study.

Consent

The PI and/or senior research team member will interview each subject and obtain informed consent from interested participants, after the participant has the study explained to them in detail and has had any questions answered.

Subject Protection

While none of the subjects in this study will be drawn from a population vulnerable to coercion or undue influence, the following measures to ensure subject protection will remain in place. Participants will be extensively screened before being accepted into the study. We will exclude participants with a history of immune, cardiovascular, pain-related, gastrointestinal, or significant psychiatric disease, or with significant allergies or Raynaud's Syndrome or a history of substance abuse. Participant screening will include blood tests for CBC and differentials, as well as thyroid hormone, and blood glucose; urinary toxicology screens will also be conducted. We will exclude

participants who smoke or take regular medications other than hormonal birth control medication. The medical history and physical will be conducted by the study physician. Should participants need medical assistance, the monitoring research assistant will notify the ward nurses, who will address any immediate medical needs and also immediately notify the study physician and the PI. In the event of a medical emergency, the CRC is located in a critical care hospital, and a code team is available at all times.

If a participant reports to have suicidal ideation (positive answer to item 9 of the Becks Depression Inventory), the physician will be called to evaluate the condition of the subject which may result in bringing the subject to the emergency room.

B7. STUDY LOCATION

Privacy and Physical Setting

Every effort to maintain subject privacy will be made. When interested participants contact the study office, a research assistant will return their call from a private office, in order to maintain confidentiality. Emails sent to and received by study participants will be based on a study email address, and direct mail will only be sent with the participant's express permission and provision of a physical mailing address. Participants will only be contacted by phone at the number they provide to study staff.

For their screening visits, participants will present to the CRC, a central shared clinical research floor in the main part of the hospital. Presence in this area of the hospital does not provide any specific information for an outside observer who witnesses the participant presenting for screening. All in-person screening, including collection of physical data from participants, will take place in a private room, behind closed doors, in the CRC. The in-hospital portion of the study will be conducted in a private or shared room within the CRC).

Whenever possible, participant information will be identified only with a 'participant identification number' and not with actual names. The exception to this is clinical data acquired in the CRC, which, in accordance with hospital policy, is identified with first and last name. As soon as it is feasible, this name identification is converted to a numeric identifier to maximize confidentiality.

B8. DATA SECURITY

All possible precautions will be taken to ensure data security. Data collected from participants will be identified using a subject number, rather than a name, whenever possible. All patient hard copies of materials containing patient information will be stored in a locked file cabinet in a locked office on a secured (swipe-card access) research floor of the hospital. Electronic data will be maintained on a secure server behind the BIDMC firewall. All computers in the laboratory require use of a login before accessing any information, and data are encrypted. Similar to hard copy materials, laboratory computers are located in locked rooms on a secure (locked) research floor of the hospital.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? Yes No

Is the BIDMC PI the lead investigator of the multi-site study? Yes No

B10 Dissemination of Research Results

Research subjects who express the desire to receive information about their individual study results or study progress will be provided with the following information: (1) Blood screening results provided by the study physician. (2) Information on sleep screening night provided and explained by PI or senior co-investigator. (3) Copy of journal publication. In addition, participation of research subjects will be acknowledged in data presentations and journal publications.